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A hydrogel matrix prolongs persistence and promotes specific localization of an oncolytic adenovirus in a tumor by restricting nonspecific shedding and an antiviral immune response

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ABSTRACT

Currently, intratumoral injection of an oncolytic adenovirus (Ad) is the conventional administration route in clinical trials. Nonetheless, the locally administered Ad disseminates to the surrounding nontarget tissues and has short biological activity due to immunogenicity of Ad, thus necessitating multiple injections to achieve a sufficient therapeutic index. In the present study, a tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-expressing oncolytic Ad (oAd-TRAIL) was encapsulated in a gelatin hydrogel (oAd-TRAIL/gel) to enhance and prolong antitumor efficacy of the virus after a single intratumoral injection. oAd-TRAIL/gel showed greater antitumor efficacy than naked oAd-TRAIL did due to enhanced and prolonged intratumoral accumulation of Ad up to a 20-day period, showing potent induction of apoptosis and inhibition of tumor cell proliferation. Furthermore, the gel system effectively prevented shedding of oncolytic Ad from the injection site to hepatic and other healthy tissues. oAd-TRAIL/gel treatment resulted in a markedly weaker antiviral immune response against Ad relative to naked oAd-TRAIL, further contributing to prolonged persistence of the oncolytic Ad in tumor tissue. Moreover, the hydrogel matrix preserved oAd-TRAIL's ability to induce an antitumor immune response, resulting in higher intratumoral infiltration by $CD4^+$/$CD8^+$ T cells. Taken together, these findings show that single intratumoral administration of the Ad/hydrogel modality may prolong and potentiate the therapeutic efficacy of Ad, modulate the immune reaction in favor of the virotherapy, and enhance intratumoral localization of the virus, ultimately overcoming limitations of oncolytic virotherapy revealed in recent clinical trials.

1. Introduction

An oncolytic adenovirus (Ad) is a promising candidate for cancer gene therapy because of several advantages, such as high gene transfer efficiency into both dividing and nondividing cells, no risk of insertional mutagenesis, production at a high titer, selective propagation in cancer cells, and efficient oncolysis by viral replication [1–3]. Furthermore, an oncolytic Ad-mediated antitumoral immune response has been shown to induce systemic antitumor activity [4–6]. Despite these therapeutically advantageous attributes, oncolytic Ads in clinical trials have shown limited therapeutic efficacy owing to induction of an Ad-specific host immune response, short duration of therapeutic gene expression, and poor persistence of Ad in a target tissue, ultimately necessitating repeated administration and high doses [7–9].

For this reason, nanomaterial-mediated delivery of an oncolytic Ad is a promising approach to evasion of immune system-mediated rapid clearance and to prolonging biological activity of the virus *in vivo* [7,10–16]. Among several types of nanomaterial-based delivery systems, a hydrogel is particularly promising for local sustained delivery of various therapeutics [8,17]. A hydrogel, which is formed by self-assembly or crosslinking of polymers, can function as a reservoir for therapeutic agents due to highly porous and hydratable structure, ultimately enabling a high concentration of therapeutics to be retained in the target tissue over an extended period [18,19]. Furthermore, the hydrogel can protect its cargo from a hostile host environment [20]. In line with these results, an oncolytic Ad loaded into a protective alginate gel has been shown to

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retain its biological activity and is released into the tumor tissue over an extended period, ultimately showing potent antitumor efficacy through enhanced virus accumulation, persistence, and distribution in tumor tissue [8].

Gelatin, which is derived from collagen, is already widely used in cosmetics and food products, as well as in pharmaceutical and medical applications because of GRAS (Generally Regarded As Safe) status issued by the United States Food and Drug Administration [21]. Furthermore, gelatin is biodegradable, biocompatible, and nonimmunogenic, making it an attractive candidate for sustained delivery of therapeutics [22,23]. Additionally, a gelatin-based delivery system is responsive to a tumor microenvironment because collagenase, gelatinase A (MMP-2), and gelatinase B (MMP-9) are overexpressed in a wide range of solid tumors, leading to enzymatic degradation of gelatin [22]. In the present study, we applied a biodegradable biocompatible gelatin hydrogel to encapsulate a tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-expressing oncolytic Ad (oAd-TRAIL), ultimately aiming to induce a sustained antitumor effect by effectively preserving biological activity of the oncolytic Ad and releasing the virus into tumor tissue through enzymatic degradation of the hydrogel.

Because recent reports highlighted the importance of an oncolytic virus-mediated antitumor immune response for inhibition of tumor growth [4,5,24–26], our primary aim in this study was to test whether hydrogel-mediated delivery of the oncolytic Ad can reduce the antiviral immune response while preserving the virus' ability to induce an antitumor immune response. To achieve this goal, antiviral and antitumor immune responses induced by the hydrogel-based oncolytic Ad delivery system were evaluated in an immunocompetent and Ad-permissive Syrian hamster model that supports viral replication, viral replication-mediated tumor cell lysis, and immunomodulation.

2. Materials and methods

2.1. Cell lines and cell culture

All growth media were supplemented with 10% of fetal bovine serum (FBS; GIBCO-BRL, Grand Island, NY), 1% of a penicillin-streptomycin solution (final 100 U/mL), and gentamycin (50 μg/mL). A hamster pancreatic carcinoma cell line (HaP-T1), hamster leiomyosarcoma cells (DDT1-MF-2), hamster normal fibroblasts, and human non-small lung carcinoma cells (A549) were cultured in Dulbecco's modified Eagle's medium (DMEM; GIBCO-BRL). A hamster kidney cell line (HaK) was cultured in the Minimal Essential Medium (MEM; GIBCO-BRL). All the cells were maintained at 37 °C in a humidified atmosphere at 5% CO_2. The cell lines (DDT1-MF-2, A549, and HaK) were purchased from American Type Culture Collection (ATCC, Manassas, VA). Hamster normal fibroblasts were isolated from hamster dermal skin by primary culture. HaP-T1 cells were kindly provided by Dr. Masato Abei (University of Tsukuba, Ibaraki, Japan).

2.2. Virus preparation

A green fluorescence protein (GFP)-expressing replication-incompetent Ad (dE1/GFP) and control oncolytic Ad (oAd) replicating under the control of modified human telomerase reverse transcriptase (TERT) promoter [27] and secretable trimeric TRAIL-expressing variant (oAd-TRAIL expresses TRAIL from Ad E3 region), which can induce the apoptosis selectively in cancer cells, were used. All replication-incompetent Ad was propagated in 293A cells, whereas oncolytic Ads were propagated in A549 cells, and purified by CsCl gradient centrifugation. The number of viral particles (VP) was measured from optical density at 260 nm (OD_{260}), for which an absorbance values of 1 is equivalent to 1.1×10^{12} VP/mL.

2.3. Preparation of an Ad-loaded gelatin hydrogel and a quantitative release experiment

Synthesis of *in situ* cross-linkable gelatin hydrogels using gelatin-hydroxyphenyl propionic acid (GHPA) was described in a previous study [23]. A 3% w/v solution of GHPA was prepared in phosphate-buffered saline (PBS) and incubated at 37 °C until it dissolved completely. Ad (5×10^8 VPs; 2.0 μL of a 10^{12} VP/mL suspension) was mixed with a 3% GHPA solution in PBS (43 μL). The 3% GHPA/PBS solution containing Ad was mixed with 0.05% horseradish peroxidase (HRP; 5 μL) and then either 0.035% or 0.14% H_2O_2 (5 μL) was mixed with the 3% GHPA/PBS solution (45 μL). HRP was added to Ad/GHPA, and this suspension was mixed with H_2O_2/GHPA by pipetting three or four times in a mold for gelation. After gelation for 20 min, the gel was transferred to a 12-well plate, and then 1 mL of PBS was added to the gel. To study the release of Ads from the gel during degradation in an enzymatic reaction, the mixture was treated with various concentrations of collagenase (0-25 mg/mL). The samples treated with collagenase were incubated at 37 °C in a humidified atmosphere containing 5% of CO_2 and 95% of air. Ad released from the Ad/gel complex was obtained from the supernatant after 24 h of collagenase treatment. Released VPs were detected by quantitative real-time PCR (Q-PCR; Applied Biosystems, Foster City, CA) as reported elsewhere [8]. To examine the burst size of the virus released from Ad/gel complex, Ad (5×10^8 VPs) was loaded into the Ad/gelatin hydrogel (50-Pa) as mentioned above. After gelation for 20 min, the gel was transferred to a 12-well plate and then 0.01 mg/mL of collagenase was added to the gel. The samples were then incubated at 37 °C in a humidified atmosphere containing 5% of CO_2 and 95% air. The supernatant was harvested at every two days, and the quantity of released Ad were analyzed by Q-PCR described above.

2.4. Assessment of cancer cell-killing efficacy

HaK, HaP-T1, or DDT1-MF-2 cells or hamster normal fibroblasts were seeded in 96-well plates and infected with either oAd or oAd-TRAIL [multiplicity of infection (MOI): 1-500]. At 48 h post infection, 200 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT; Sigma Chemical, St. Louis, MO) in PBS (2 mg/mL) was added into each well. After 4 h incubation at 37 °C, the supernatant was discarded, and formazan was solubilized with 150 μL of dimethyl sulfoxide. Absorbance was measured on a microplate reader at 540 nm.

2.5. A viral production assay

HaK cells were seeded in a 12-well plate at 5×10^4 cells/well and infected at MOI of 5 with oAd or oAd-TRAIL. The medium was removed at 4 h postinfection and replaced with a fresh one containing 5% of FBS. The supernatants and cells infected with oAd or oAd-TRAIL were harvested at 4, 24, 48, or 72 h after the infection. The copy number of viral genomes was determined by Q-PCR (Applied Biosystems) as previously reported [8].

2.6. The antitumor effect in a hamster model

Syrian golden hamsters (Japan SLC Inc., Tokyo, Japan) were maintained in a laminar airflow cabinet under specific pathogen-free conditions. All the facilities have been approved by the